FIELD CLINIC PROCEDURES FOR DIAGNOSIS OF ECHINOCOCCUS GRANULOSUS IN DOGS¹

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ABSTRACT.—*Echinococcus granulosus* is the causative parasite of hydatid disease in humans and represents a significant public health problem within endemic foci in all major continents of the world. This report gives a detailed set of instructions whereby four trained individuals can examine 15–20 dogs per hour for the presence of this organism. The procedure permits the baseline determination of the prevalence of this parasite within any specific population of dogs and also allows the periodic examination of the same animals to determine if recommended preventive and control measures for hydatid disease are being followed by sheep and dog owners in any region where the parasite is known to occur.

Echinococcus granulosus is an extremely small tapeworm (4-6 mm in length; Fig. 1)that lives in the small intestine of dogs and a few related carnivores (e.g., covotes and wolves). Eggs from the fully developed tapeworm are passed out with the fecal material from the carnivore host. Sheep (and a variety of other domestic and wild animals such as cattle, pigs, deer, and moose) may ingest vegetation contaminated with the carnivore host feces containing these tapeworm eggs. Once the eggs have been ingested by these animals (intermediate hosts), the tapeworm eggs hatch in the duodenum, penetrate through the intestinal lining, and pass via the bloodstream to such filtering organs as the liver or lungs. There the hatched eggs undergo development to the larval stage (termed hydatid cysts; Fig. 2) and become filled with watery (hydatid) fluid. The hydatid cysts continue to grow inside these animals, and tiny microscopic tapeworm heads (termed protoscolices; Fig. 3) develop inside the cysts by extensive asexual reproduction. Once an animal is infected with these hydatid cysts, it has them for the remainder of its life. When that animal dies or is killed, the viscera with the hydatid cysts may be eaten by a dog or other carnivore. The protoscolices are then liberated from the cyst, attach to the intestinal lining of the carnivore, and develop to the tapeworm stage (Schantz 1982). The life cycle of E. granulosus is given in Figure 4.

Developmental time in the dog after it eats viscera containing hydatid cysts from an infected sheep until mature tapeworms can be found in the dog's intestine is about 35 days (Thompson 1986). Developmental time in the sheep after it ingests vegetation contaminated with fecal material from the dog containing tapeworm eggs until mature hydatid cysts with protoscolices can be found in the sheep viscera is approximately one year (Schantz 1982).

People who work in close association with dogs and sheep that harbor this tapeworm are also at some risk of contacting the parasite. If such individuals inadvertently ingest some of the tapeworm eggs passed from an infected dog (either from petting or handling the dog or from ingesting food or drink contaminated with dog feces), hydatid cysts may eventually develop within the internal organs of that person. Such an infected person is said to have hydatid disease or echinocoecosis. The cysts will continue to grow and develop and may become so large as to interfere with the normal functioning of the particular organ (liver, lung, etc.) in which the cysts are located. Although there are several chemical compounds that will effectively retard the growth of hydatid cysts in humans, there are no compounds that will remove or eliminate the cyst entirely. Consequently, the cysts must on occasion be removed through surgery. Such an operation is naturally very serious, depending

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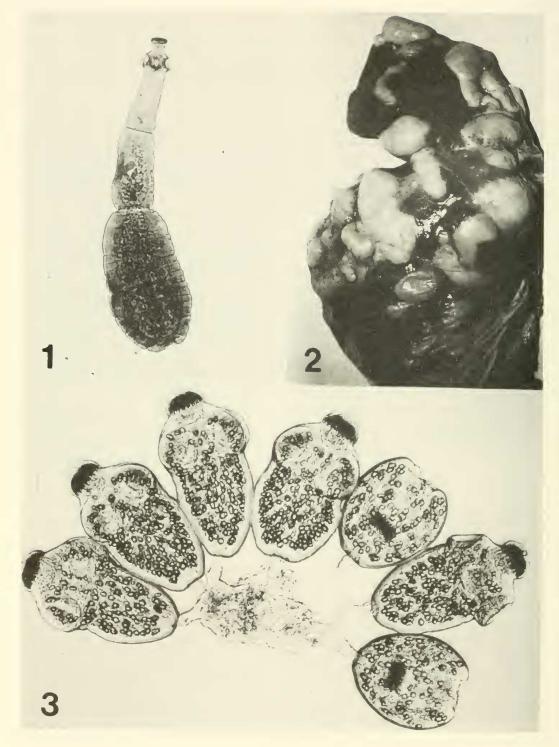


Fig. 1. Adult *Echinococcus granulosus* tapeworm from an infected dog. Fig. 2. Hydatid cysts in liver from an infected sheep. Fig. 3. Protoscolices from a hydatid cyst.

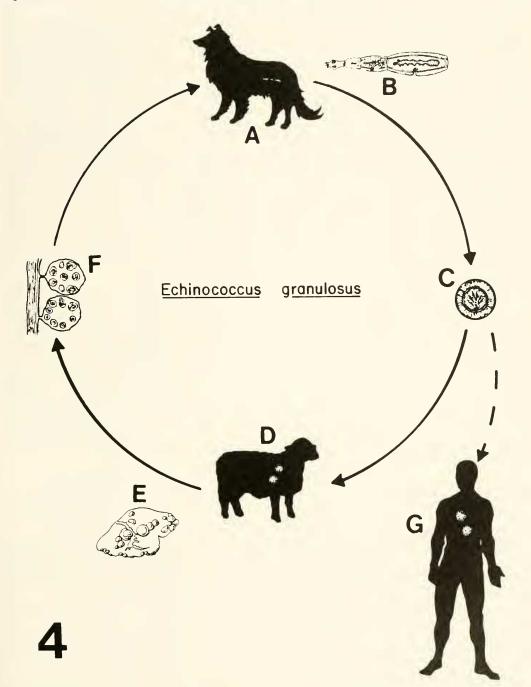


Fig. 4. Life cycle of *Echinococcus granulosus*: A, Dog (carnivore host) infected with *Echinococcus granulosus* tapeworm in small intestine; this animal becomes infected after eating viscera of sheep (or a related animal) containing hydatid cysts; B, Adult *Echinococcus granulosus* tapeworm (4–6 mm) in small intestine of dog; C. Tapeworm egg (30–40 μ) passed in feces from an infected dog; D, Sheep (intermediate host) with hydatid cysts in viscera; this animal becomes infected after ingesting vegetation contaminated with dog feces containing tapeworm eggs; E, Hydatid cysts in viscera of sheep: F, Tissue section through hydatid cyst with daughter cysts and numerous protoscolices (tiny tapeworm heads); G, Human with hydatid cysts in liver and lung; people become infected after ingesting food or drink contaminated with dog feces containing tapeworm eggs.

upon the size and specific location of the developing cyst(s), and in rare cases it may be fatal (Schantz 1982).

At the present time hydatid disease is known to be endemic in parts of Europe, Asia, Africa, South and Central America, New Zealand, and Tasmania. Although the disease is relatively rare in North America, known endemic foci do exist in such places as Alaska, Utah, Arizona, New Mexico, and the Central Valley of California (Gemmell 1979, Andersen 1986).

In many countries of the world where hydatid disease is known to be a significant problem, surveillance studies are routinely done to determine the prevalence of Echinococcus granulosus in people, sheep, and dogs (Barbour et al. 1978, Condie et al. 1981, Andersen et al. 1986). Data for the prevalence of hydatid cysts in people come mainly from a survey of hospital records. Data for the prevalence of hydatid cysts in sheep are obtained most often from records at slaughter houses or from a survey of sheep owners who may have observed hydatid cysts in sheep they have killed. However, information on the prevalence of E. granulosus tapeworms in dogs is more difficult to obtain. As stated above, these particular tapeworms are extremely small and are not seen by the dog owner or even by the veterinarian at routine inspections. They can, however, be detected by a very thorough examination of the intestinal contents after the dog is killed. This works well for examining dogs suspected of harboring this parasite if the dogs are either strays or not needed as working dogs. Obviously, many of the dogs in an agricultural region are required as working dogs for the sheep industry and, as such, cannot be killed; yet these dogs are the very ones that most likely will be infected with this particular tapeworm. In those cases, the prevalence of E. granulosus may be determined through using purgation techniques-i.e., use of a strong laxative (Gemmell 1973, Schantz 1973). Such a procedure not only allows the determination of baseline data on this parasite within dogs living in a specific region, but it also allows the periodic examination of the same animals to determine if recommended preventive and control measures for this disease are being followed by sheep and dog owners in that area. Sheep ranchers must do all they can to

prevent dogs from having access to viscera of any infected sheep that might die at their farmstead or range. Specifically, they must not purposefully feed sheep viscera to dogs when the sheep are butchered for mutton (Andersen et al. 1983).

MATERIALS AND METHODS FOR PURGING DOGS

The following information is designed as a set of recommended instructions for purging dogs at a field clinic in a rural community where sheep raising is an important part of agriculture. The specific protocol described requires four trained individuals and is designed to allow the examination of approximately 15–20 dogs per hour.

A. Initial organization.

- 1. Obtain all necessary approvals from local health officers who may need to be involved or give sanction to the clinic.
- 2. Advertise details of the clinic through: a. any local newspapers;
 - b. personal letters to dog or sheep owners where feasible;
 - c. announcements sent to schools, churches, or community centers;
 - d. posters displayed at local stores or community centers.
- 3. Details should include:
 - a. nature of hydatid disease;
 - b. its public health significance;
 - c. exact location, date, and time of field clinic;
 - d. instructions to dog owners to:
 - withhold all food from their dog for 12 hr before the examination (water should be continually available, however);
 - (2) bring each dog with a sturdy leash;
 - (3) be prepared to sign a "release of responsibility" form for the examination team.
- 4. Select a site for the clinic somewhat removed from any residential area, playground, public school, or major traffic region. The area where the dogs are to be tied should be relatively free of tall grass, bushes, and any other objects that would inhibit the eventual collection of purged fecal samples. Generally, it is best to avoid the use of

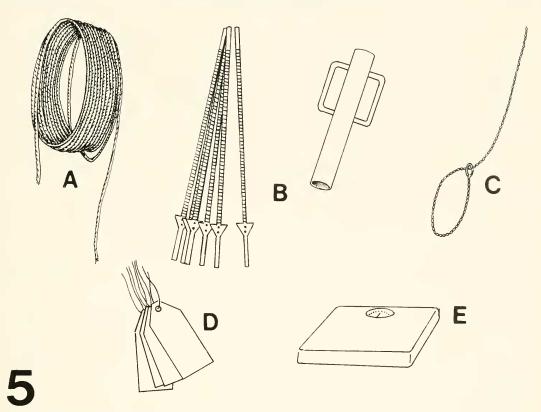


Fig. 5. Materials for dosing line: A, Long, sturdy rope; B, Metal stakes and stake driver; C, "Choke chain" type leash; D, Identification tags; E, Scales.

cement foundations, paved lots, or even graveled roads. A relatively firm soil substrate nearly free of vegetation seems to be the best type location for a field clinic.

B. Preparation on day of clinic.

- 1. All members of the examining team should arrive at the clinic site in sufficient time to be completely organized before owners start to bring dogs for examination. The four members of the team should be assigned to separate duties:
 - a. No. 1 interviews owners and registers all dogs.
 - b. No. 2 administers all purgative medicine.
 - c. No. 3 collects all purged samples.
 - d. No. 4 examines all samples.
- 2. A good sturdy fence which dogs cannot jump over or climb through is the best place to tie the individual dogs. If a good fence is not available, a temporary

"dosing line" can be constructed with metal posts and a long, heavy rope. Dogs can be tied about 2.5 m apart on a very short leash. This will minimize fighting among the dogs and will lessen any confusion as to which fecal samples belong to which dog. The rope, stakes, stake driver, leashes, individual tags, and scales are shown in Figure 5.

3. All members of the examination team should wear protective clothing (Fig. 6), with the exception of the individual who is assigned to interview the dog owners. That person should not wear a mask or use gloves so that he or she can communicate easily with those who attend the clinics and can also handle all records and educational aids. The wearing of protective clothing serves to protect the members of the examination team and also emphasizes to those who attend the clinic the potential seriousness of hydatid disease.

GREAT BASIN NATURALIST

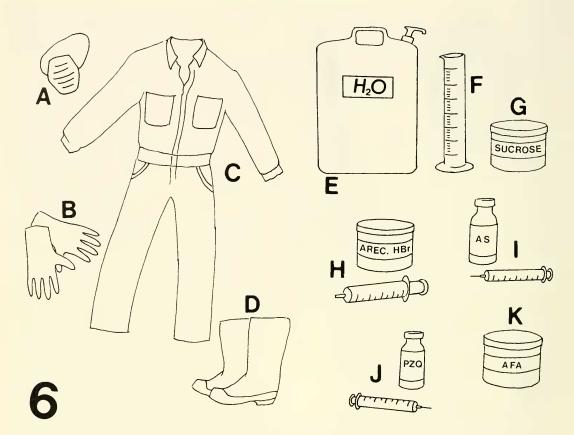


Fig. 6. Protective clothing, chemicals, drugs, and miscellaneous solutions: A, Face mask; B, Latex disposable gloves; C, Coveralls; D, Boots; E, Water container; F, Graduated cylinder; G, Sucrose; H, Arecoline HBr (purgative) and syringe without needle; I, Atropine sulfate (antidote) and syringe with needle; J, Praziquantel (therapeutic drug) and syringe with needle; K, AFA tapeworm preservative solution.

4. A disposal pit into which collected fecal material and disposable items and supplies can be placed should be dug in close proximity to the examination site. The pit needs to be about the size and depth of a regular 30-gal garbage can. If a disposal pit cannot be dug at the clinic site, a large garbage can fitted with a sturdy plastic liner should be available.

C. Registration of dogs.

- 1. As the dogs arrive, one member of the examining team is assigned to greet and interview each owner to obtain the following information:
 - a. name and address of dog owner;
 - b. name, age, sex, breed, any identifying features, and weight of dog (owner can hold dog on scales and then subtract own weight without dog);

- c. history of the dog's use in agriculture, including answers to the following questions:
 - (1) Does dog have contact with sheep?
 - (2) Does owner have sheep? If so, how many?
 - (3) Does owner allow dog to eat sheep viscera?
 - (4) Has dog been treated within the past year for tapeworms?
- 2. Owner is requested to read and sign a "release of responsibility" form (Fig. 7) that releases all members of the examination team from any and all financial obligation should the dog be injured or die as a result of the purgation or subsequent treatment.
- 3. Owner is then given educational aids concerning the nature and transmissi-

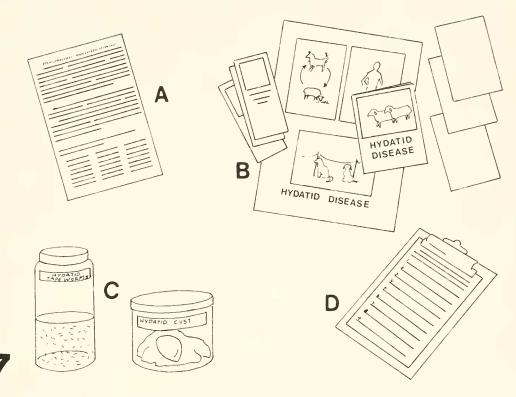


Fig. 7. Registration forms, educational materials, and survey record sheets: A, Registration data form (top portion), release of responsibility statement (middle portion), results of examination for all dogs belonging to one owner (bottom portion); B, Educational materials for those who attend clinic; C, Preserved specimens of *Echinococcus granulosus* tapeworms from an infected dog and hydatid cysts from an infected sheep; D, Clipboard and record sheets for all dogs examined at the clinic.

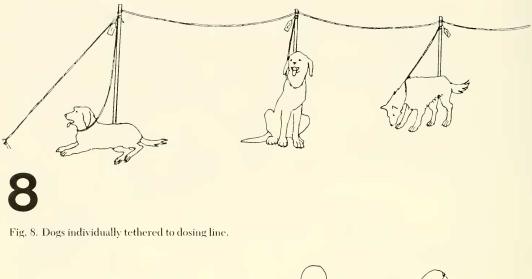
bility of hydatid disease and is shown the sample of preserved *Echinococcus* granulosus tapeworms from an infected dog and the sample of a preserved hydatid cyst from an infected sheep (Fig. 7).

 The dog is then taken to the dosing line where it is individually tethered (Fig. 8). An identification number is given to each dog as it is entered onto the line, and that number is placed on all registration forms and the master list for that particular clinic.

D. Administration of the purgative solution.

1. Arecoline HBr is the purgative agent used and should be premixed as follows: 1.5 g of drug added to 100 ml of water (Fig. 6). Also, sucrose (about 15 g) is added as a sweetener to remove the unpleasant, bitter taste of the compound. The addition of a sweetener is especially important if the dog might need to receive more than one dose on the day of the clinic or if it will be brought back to another clinic at a later date.

2. A veterinarian or one specifically trained individual on the examining team should be assigned to administer all purging medicine at any one clinic. If a dog is tame and manageable, this person can probably give the purgative without help from an assistant (Fig. 9A). If, however, the dog is somewhat unmanageable, it is best for the owner or another member of the team to hold the dog firmly as shown in Figure 9B while the first person administers the drug. Arecoline HBr is administered at a dosage level of 1 ml/4.5 kg (10 lbs) of body weight. The drug should be quickly deposited at the back of the tongue to facilitate swallowing. The mouth of the dog is quickly closed, the



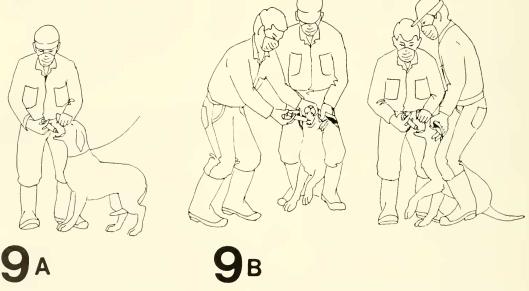


Fig. 9. Illustration of purging technique: A, One-man procedure for manageable dogs; B, Two-man procedure for unmanageable dogs.

muzzle elevated somewhat, and the attendant should make sure that the dog swallows the entire dosage amount. If an unmanageable dog is restrained by the owner or second assistant, that person *must not* release the head of the dog until the person administering the drug has pulled away from the dog's mouth. Every effort should be made to handle the dogs gently but firmly. Tight restraint should be used only when necessary, and a good practicing veterinarian should be able to dose most of the dogs single-handedly.

3. The time when each dog receives arecoline is recorded on the identification tag and also entered onto the individual registration form. Shortly after the compound is administered, most dogs will begin to salivate heavily and will also usually vomit. This material should be collected readily and discarded into the disposal pit or garbage container. Some dogs may show moderate to severe reactions to the arecoline HBr and may exhibit marked distress, cardiac excitation, convulsion, and collapse. Generally, such reactions are only temporary. In persistent cases, however, the veterinarian (or person in charge of dosing with arecoline) must be prepared to administer an antidote of atropine sulfate. This is given intramuscularly or subcutaneously at a dose rate of 0.05 to 0.1 mg/kg (Fig. 6). This antidote should
examiner is not satisfied with previous collections.
If, after approximately 30 minutes following administration of the arecoline HBr, a particular dog has not purged and shows no signs of inner peristaltic distress, the attendant might exercise the dog with a short walk in the vicinity of the dosing line. Some dogs are extremely reluctant to defecate while being tethered, and the exercise might be an added stimulus to the purgation process.
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If after 45 minutes or so there has been no purgation whatsoever, the attendant veterinarian might elect to give a second purge (about one-half the initial level). In very rare instances, even a third dose might be given if the veterinarian deems the dog to be in sufficient health and constitution to withstand such a regimen.

- 3. When a good sample with mucus is passed, one attendant carefully collects the material, labels the collecting cup, and takes the container to a central location for examination (Fig. 10). To minimize any record-keeping errors and to maintain consistency in examination procedures, one member of the team is assigned to do all examination for that particular clinic.
- 4. Several ml of tap water are added to the collecting cup from a squeeze bottle, and the material is carefully poured into a shallow black-bottom pan for examination. The attendant carefully separates the collected sample with a teasing needle and methodically examines the material with a gentle swirling motion of the pan. The examination should be done in ample lighting, which gives a good color contrast of the tiny white tapeworms against the black background of the examination pan. In cases where objects are difficult to differentiate, the object in question can be viewed under a hand lens or transferred with a medicine dropper to a petri dish and examined in greater detail under a dissecting microscope (Fig. 6). Extreme care must be taken to obtain adequate purged samples and then view them with consistency to locate

erate to severe reactions to the arecoline HBr and may exhibit marked distress, cardiac excitation, convulsion, and collapse. Generally, such reactions are only temporary. In persistent cases, however, the veterinarian (or person in charge of dosing with arecoline) must be prepared to administer an antidote of atropine sulfate. This is given intramuscularly or subcutaneously at a dose rate of 0.05 to 0.1 mg/kg (Fig. 6). This antidote should allow the dog to recover rapidly; however, it will also probably stop the purgation reflex and that particular dog will then need to be released from the dosing line without further examination. Pups under four months, extremely old dogs, and pregnant or lactating female dogs should probably not be purged (Andersen 1986). In addition, it has been our experience in holding clinics in central Utah over the past 15 years that the small "toy breeds" are likely to show adverse reactions to an arecoline purge.

E. Collection and examination of purged samples.

 In most circumstances when the purgation process proceeds normally, the dog will first void solid to semi-solid fecal material. Since this portion rarely contains parasites, it should be collected from the ground immediately and discarded. After a short delay the second purged material should be a much more liquified portion with small moderate amounts of mucus present. This portion (especially any mucus) should be carefully picked from the soil substrate with a tongue depressor and transferred to a labeled collecting cup. It is helpful if one attendant holds the dog by the leash to one side while the other attendant collects the purged sample. Additional purged amounts may be passed from the dog while it is tied to the dosing line. This material may be collected and examined also if time is available and if the

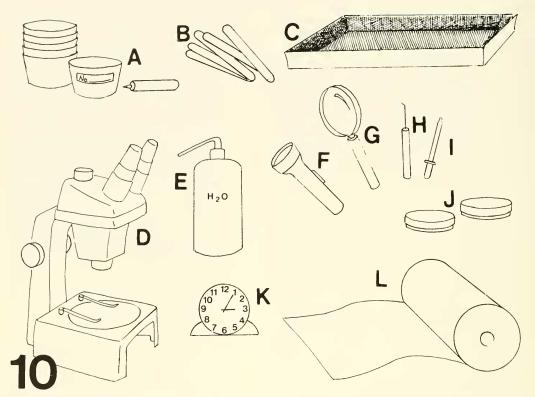


Fig. 10. Examination supplies: A, Collecting cups and marker; B, Tongue depressors; C, Shallow black-bottom examination pan; D, Dissecting microscope; E, Water squeeze bottle; F, Flashlight; G, Hand lens; H, Teasing needle; I, Medicine dropper; J, Petri dishes; K, Clock; L, Roll of paper.

the tiny worms if indeed they are present. Care must also be taken to avoid misidentification of tiny white objects that might have an overall shape similar to the *Echinococcus* tapeworms. Broken or isolated scolices (tapeworm heads) or single proglottids (tapeworm segments) are extremely difficult to detect and differentiate from extraneous materials of similar size and shape.

- 5. The results are recorded for each dog on the individual registration form and also on the master record for that day's clinic. A record should be kept of:
 - a. the quality of the purge (i.e., good, fair, poor);
 - b. presence of other worms (i.e., ascarids, large taeniids, etc.; these can be preserved and identified at a later time if that is part of the project protocol since such information is helpful in assessing the eating habits of the dogs);

- c. presence of *Echinococcus granulo*sus.
- F. Anthelmintic treatment.
 - 1. Any dog shown to be infected with *Echinococcus granulosus* must be treated before the dog is taken from the dosing line. Injectable praziquantel (PZQ) at a dose level of 5 mg/kg is recommended (Andersen et al. 1978). If the program is so designed, all dogs brought to the clinic (irrespective of whether or not they are found to harbor tapeworms) can be treated with the tapeworm medication (Fig. 6).
 - 2. All treatment given should also be noted on the individual registration forms.

G. Removal of dogs from dosing line.

 As soon as a particular dog is finished, it should be removed from the dosing line, all purged fecal material should be collected and discarded, and a new dog entered onto that site on the dosing

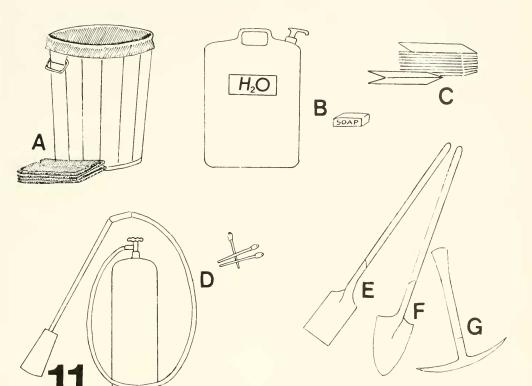


Fig. 11. Clean-up materials: A, Garbage can and plastic liners; B, Water and hand soap; C, Paper towels; D, Propane burner and matches; E, Flat-bladed shovel; F, Round-bladed shovel; G, Hand pick.

line. Dogs that are removed may have rather soiled hindquarters and may need to be eleaned somewhat before leaving the area. It is important to keep the animal as elean as possible while it is on the dosing line and not to permit it to lie down in purged material. Since the dog may purge additional amounts after it has left the clinic site, and since any tapeworm eggs passed from a treated animal are probably not killed by praziquantel (Thakur et al. 1979), it is important that the dogs not be confined near the family home for one to two days following purgation. Owners should also be told of the significance of the results of the examination and be allowed to ask questions concerning the clinic. Members of the examining team should avoid using technical words not understood by dog owners or by other interested individuals who attend these field clinics.

H. Clean-up at clinic site.

- 1. Figure 11 shows the materials and supplies neeessary for proper clean-up following the field clinic. After all dogs have been removed from the dosing line, all fecal material remaining should be collected with a flat-bladed shovel and discarded in the disposal pit or garbage container. A propane weedburner or flame-thrower is then used to heat the area where the dogs have been tethered. Shovels and other equipment used by clinic personnel can be washed clean over the disposal pit and then flamed with the burner as well. If a temporary dosing line has been used, the rope should be recoiled without allowing it to get in the dirt, and the stakes should be carefully removed and reloaded into the team vehicle.
- 2. The individual assigned to keep all registration forms and all records should not wear gloves or mask during the

clinic and should refrain from handling any potentially contaminated material. This individual should be responsible for putting away all records, visual aids, and all other materials that have not been handled by those individuals wearing gloves at the clinic.

3. Coveralls should be removed and placed in a plastic bag and should not be worn again without first being boiled in water. Gloves and masks should be discarded, and all team members should wash their hands carefully with soap and water and dry with disposable paper toweling. All other disposable items from the clinic should be discarded into the disposal pit, which should then be covered with an adequate amount of soil to prevent any dogs (or children) from digging into the buried material. If a large garbage can is used instead of a disposal pit, the plastic liner should be tied securely and then eventually incinerated or buried at another site. It is virtually impossible to describe each step of precaution that should be taken by the examination team, but each member should be expressly concerned about his or her own safety as well as that of the other members of the team.

DISCUSSION

Arecoline HBr is a drug manufactured originally from the areca nut which was used by the ancient Chinese for removal of intestinal worms. It was first used against tapeworms in dogs in 1921 (Schantz 1973). However, with the advent of newer, more effective anthelmintics, the use of arecoline HBr in dogs has recently been limited to that of a diagnostic compound. The drug first eauses the tapeworms to relax and lose their attachment to the intestinal mucosa; it then causes a marked contraction of the intestinal smooth muscles of the dog (Munday and Smith 1972). This results in an expulsion (purgation) of some or many of the intestinal worms in an infected animals. The compound is known to remove about 90% of all tapeworms present in infected dogs in less than one hour after administration, about half of the ascarid worms, but none of the hookworms (Batham 1946).

Arecoline is used today in many parts of the world in areas where hydatid disease is known to occur as an integral part of preventive and control programs in which purging of dogs for detection of any *Echinococcus* tapeworms present is coupled with health education, control of livestock slaughtering, and improved management of high-risk dogs (Schantz 1982). In central Utah the use of arecoline in field clinics has aided in the overall decrease of Echinococcus in infected dogs from a prevalence of 28.3% in 1971 (Andersen et al. 1983) to 2.3% in 1984 (Andersen et al. 1986). This decrease substantiates the benefit of incorporating arecoline purging into a control program for hydatid disease. Dog owners can see first hand if their dogs are indeed infected with these important parasites, which then gives immediate reinforcement to the overall program. Unfortunately, in one study in central Utah 92.5% of the dog owners surveyed knew the cause of hydatid disease and how the parasite was transmitted, 90% of them knew someone who had had surgical removal of hydatid cysts, and yet nearly half of the respondents indicated they still allowed their dogs to sometimes eat part of the sheep carcass following routine butchering on their premises or in the fields (Schantz and Andersen 1980).

An important additional point for workers to remember where arecoline is used as a purging agent in dogs is that varied adverse effects such as tremors, difficulty in breathing, incoordination, and possible collapse can sometimes occur in dogs given this compound (Forbes and Whitten 1961). Also, some owners have complained that their dogs have been definitely weakened and were unable to work in the livestock industry for at least one day following purgation (Batham 1946).

As discussed earlier, the actual examination for the tiny *Echinococcus* tapeworms is very difficult and is best left to experienced individuals. Otherwise, false negative results may be recorded that would lead to improper confidence in the particular control program. In *Echinococcus* diagnostic field elinics, the use of arecoline in the hands of less-than-capable individuals may not only be useless but may even be dangerous if not carried out by experienced personnel and in a standardized manner (Sehantz 1982).

In summary, the use of diagnostic field clinics for detection of *Echinococcus* tapeworms is April 1987

best coupled with an intensive educational effort and with improved management programs by all sheep and dog owners living in endemic regions (Crellin et al. 1982). Following the initial determination of baseline data on the prevalence of *Echinococcus* tapeworms, periodic clinics thereafter with these same high-risk sheep dogs will provide the necessary index of progress which health authorities need to continue direction of successful campaigns in endemic regions.

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